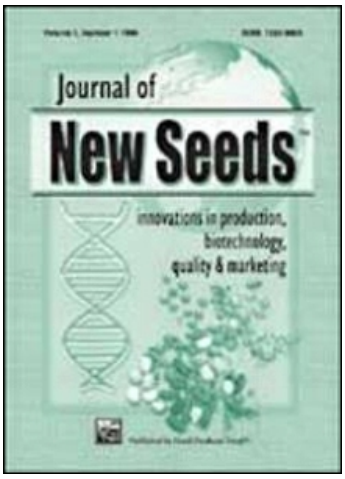

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The Effects of Fusarium thapsinum, Curvularia lunata, and Their Combination on Sorghum Germination and Seed Mycoflora

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The Effects of *Fusarium thapsinum*, *Curvularia lunata*, and Their Combination on Sorghum Germination and Seed Mycoflora

Louis K. Prom

ABSTRACT. Grain mold, caused by several species of fungi, reduces grain yield and quality. This study examined the effect of the two most common grain mold fungi, *Fusarium thapsinum* and *Curvularia lunata*, inoculated singly and together on germination and seed mycoflora. Germination rates for the different cultivars have been reported in details previously. *C. lunata* was the most frequently isolated fungal species followed by *F. thapsinum* and *F. semitectum*. Non-inoculated control samples had *Alternaria* spp. and *F. semitectum* as the most frequently recovered fungal species followed by *C. lunata*. There were highly significant negative correlations between germination and *C. lunata* ($r = -0.44$ and $P < 0.01$) in 2000 and between germination and *F. thapsinum* ($r = -0.52$ and $P < 0.01$) in 2001. Significant negative correlations between *F. semitectum* and *F. thapsinum*, and highly significant negative correlations between *F. thapsinum* and *C. lunata* were observed. Data from this study indicate that the fungal species present, the environment, and the sorghum cultivar all influence seed mycoflora.

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KEYWORDS. Cultivars, germination, grain mold, fungal species, mycoflora analysis, sorghum

INTRODUCTION

Grain mold, a fungal disease complex, is one of the principal constraints to sorghum productivity and grain quality worldwide. Moist conditions late in the growing season are especially conducive to disease development (Bandyopadhyay and Chandrashekar, 2000; Garud et al., 1998; Singh and Bandyopadhyay, 2000). Although, fungi in several genera have been associated with grain mold (Bandyopadhyay and Chandrashekar, 2000; Esele et al., 1995; Singh and Bandyopadhyay, 2000), *Fusarium thapsinum* Klittich, Leslie, Nelson, and Marasas and *Curvularia lunata* (Wakk.) are considered the most common pathogenic agents (Singh and Bandyopadhyay, 2000).

Seed discoloration, ranging from pink to reddish-pink, or black to grayish-black is a common visual symptom of grain mold (Castor, 1981; Singh and Bandyopadhyay, 2000). Grain mold reduces grain quality through seed deterioration. Seed development is also influenced by grain mold, which results in a reduction in seed size. Yield losses ranging from 30-100% may occur with severe epiphytotics (Singh and Bandyopadhyay, 2000). Planting photosensitive cultivars that mature during periods of dry weather or resistant cultivars can minimize these yield losses. Seed germination and seedling vigor of infected seed can be enhanced through the use of chemical treatment in the form of seed dressings (Forbes et al., 1992; Singh and Bandyopadhyay, 2000).

While significant research has been conducted on genotypic reaction to grain mold in general, there is little information on the association between seed mycoflora and germination of sorghum cultivars. Castor (1981) reported a negative correlation between seed germination and *Fusarium* spp. Forbes et al. (1989) inoculated several sorghum cultivars with *F. moniliforme* and noted negative correlations between grain mold severity and seed germination. Garud et al. (2000a) also reported a negative correlation between seed germination rates and *Fusarium* spp. Both Castor (1981) and Garud et al. (2000a) noted non-significant correlations between *Curvularia* spp. and seed germination.

This study was conducted to determine the effect of *F. thapsinum* and *C. lunata* and a mixture of the two species on seed mycoflora, seed viability, and the association between these two traits for eight sorghum cultivars.

MATERIALS AND METHODS

Field Trials

Field trials and experimental design have been described in detail (Prom et al., 2003). Briefly, experiments were established at the Texas A&M Agricultural Research Farm in College Station, Texas during 2000 and 2001. Eight sorghum cultivars were selected for this study, which have varying degrees of susceptibility to grain mold (Rodriguez-Herrera, 1999). Sureno and Dorado are grown primarily for food in Central America (Meckenstock et al., 1993). Sureno is considered resistant to grain mold and the remaining seven lines are moderately susceptible to susceptible. Cultivars RTx2536 and RTx430 are commonly used as pollinators in commercial hybrid sorghum seed production (Miller, 1984). 98LB650, 98LB711, 98LB723, and 98LB789 were selected from a recombinant inbred line population, resulting from the cross of Sureno × RTx430 (Klein et al., 2001).

A randomized complete block design with a split-plot arrangement was utilized in the study. Cultivars were main plots and treatments sub-plots. The eight cultvars were planted in 20 row plots 6 m in length with 0.31 m row spacing. Four treatments were used in both years. The fungal treatments consisted of individual panicles inoculated with *F. thapsinum*, *C. lunata*, and a mixture of *F. thapsinum* and *C. lunata*. Untreated control panicles were sprayed with sterile water. For each treatment, three panicles per cultivar were used in 2000 and four panicles per cultivar in 2001. Each treated or control panicle was considered a replicate.

Inoculation Protocol

Inoculation protocol and conidia of *F. thapsinum* and *C. lunata* used in this study have also been described in detail previously (Prom et al., 2003).

Parameters Measured

At maturity, treated and the untreated control panicles were hand harvested and threshed using a single head thresher (Almaco Plant and Head Thresher, Allan Machine Company, Ames, Iowa). Seed mycoflora and germination rates were determined for each panicle.

Germination rates were determined as described previously (Prom et al., 2003). Seed mycoflora was determined for 150 seeds/treatment. Seeds were surface disinfested prior to evaluation. Fifty seeds were placed in vials (3 vials/treatment), immersed in 10 % NaOCl for 1 min, rinsed three times in sterile distilled water, and dried under a laminar flow hood. Seeds were plated on half-strength potato dextrose agar and incubated at 25°C with a 12 hr. photoperiod for 7 d. Identification of the fungal species was based on the conidia, conidiophores, colony morphology, color, and the descriptions provided by Booth 1971, Ellis 1971, and Nelson et al. 1983. Confirmation of the *Fusarium* spp. was conducted at the Fusarium Research Center, The Pennsylvania State University.

RESULTS

The germination rates for the different cultivars have been previously reported in detail (Prom et al., 2003). Briefly, fungal treatments significantly reduced seed germination rates when compared to the water-sprayed controls on all the cultivars tested. *C. lunata* caused the most severe reduction in seed germination rates for 98LB650, 98LB711, 98LB723, RTx2536, Dorado, and Sureno in 2000. *F. thapsinum* caused the most severe reduction in seed germination rates for 98LB650, 98LB711, 98LB789, and RTx2536 in 2001.

Seed mycoflora: C. lunata was the most frequently isolated fungal species followed by *F. thapsinum*, and *F. semitectum* (Tables 1 and 2). *C. lunata* accounted for 39% and 29% of the total fungal species recovered from seeds in 2000 and 2001, respectively. In 2000, the frequency of recovery of *F. thapsinum* from seeds was 30%, *F. semitectum* 13%, *Alternaria* spp. 9%, and *Fusarium* spp. was 7% (Table 1). In 2001, *F. thapsinum* accounted for 25%, *F. semitectum* 15%, *F. chlamydosporum* 6%, *F. proliferatum* 1%, and other *Fusarium* spp. 14% of the total fungal species recovered from seeds (Table 2). *Alternaria* spp. accounted for 8% and *Bipolaris* spp. 2% of the total fungal species isolated from seeds in 2001.

In 2000, germination and *C. lunata* exhibited a highly significant negative correlation ($P < 0.01$), whereas, a highly significant negative correlation between germination and *F. thapsinum* was noted in 2001 (Table 3). All other fungal species did not negatively impact germination in this study. In both years, significant negative correlations be-

TABLE 1. Frequency of recovery (%) of various fungal species from eight sorghum cultivars inoculated with *Fusarium thapsinum* (FT), *Curvularia lunata* (CL), a mixture of the two fungi (MIX), and water-sprayed control (CON) in 2000.[1]

		Fungal species					
Line	Treatment	FT	CL	FS[2]	F spp.[3]	Alt.[4]	Others[5]
98LB650	CL	3	95	2	3	0	0
98LB650	CON	5	32	12	9	25	17
98LB650	FT	78	4	1	7	8	3
98LB650	MIX	51	33	5	5	3	3
98LB711	CL	3	90	1	4	0	1
98LB711	CON	11	28	22	13	21	5
98LB711	FT	63	6	1	23	7	0
98LB711	MIX	51	40	2	6	1	0
98LB723	CL	32	64	2	1	0	1
98LB723	CON	1	13	22	10	39	15
98LB723	FT	69	9	5	9	7	0
98LB723	MIX	81	17	1	1	1	0
98LB789	CL	0	85	11	3	0	0
98LB789	CON	4	15	44	10	25	3
98LB789	FT	65	7	19	5	5	0
98LB789	MIX	32	45	17	5	1	0
Dorado	CL	4	77	7	9	0	2
Dorado	CON	11	16	24	14	30	5
Dorado	FT	77	7	5	4	7	1
Dorado	MIX	37	39	10	10	4	0
RTx2536	CL	0	85	13	2	0	0
RTx2536	CON	3	40	24	4	21	8
RTx2536	FT	33	23	25	17	2	1
RTx2536	MIX	27	47	24	1	0	0
RTx430	CL	1	68	22	7	1	1
RTx430	CON	6	30	27	11	21	4
RTx430	FT	53	11	29	6	2	0
RTx430	MIX	32	44	13	9	3	0
Sureno	CL	1	94	5	0	1	0
Sureno	CON	7	33	13	5	39	3
Sureno	FT	75	13	1	3	7	1
Sureno	MIX	53	33	5	5	3	1
Overall Mean		30	39	13	7	9	2

[1] Frequency of recovery (%) of the various fungal species was based on assays of 150 seeds per line/treatment combination plated on half-strength potato dextrose agar medium.
[2] FS = *Fusarium semitectum*.
[3] F spp. = other *Fusarium* species.
[4] Alt. = *Alternaria* species.
[5] Others = other fungal species.

TABLE 2. Frequency of recovery (%) of various fungal species from eight sorghum cultivars inoculated with *Fusarium thapsinum* (FT), *Curvularia lunata* (CL), a mixture of the two fungi (MIX), and water-sprayed control (CON) in 2001.[1]

		Fungal species								
Line	Trt[2]	FT	CL	FS[3]	F spp.[4]	FP[5]	FC[6]	Alt.[7]	BI.[8]	Others[9]
98LB650	CL	3	47	2	1	0	44	3	0	0
98LB650	CON	1	20	33	15	0	4	20	7	0
98LB650	FT	55	4	5	11	10	4	7	3	0
98LB650	MIX	56	29	3	9	0	0	3	0	0
98LB711	CL	0	60	9	23	0	0	5	3	1
98LB711	CON	2	11	18	17	0	5	41	4	2
98LB711	FT	31	8	13	25	0	0	15	7	0
98LB711	MIX	17	33	8	35	0	0	8	0	0
98LB723	CL	5	49	11	21	0	14	0	0	0
98LB723	CON	5	12	23	39	1	0	17	3	0
98LB723	FT	77	1	3	15	0	0	5	0	0
98LB723	MIX	43	28	14	12	0	0	1	2	0
98LB789	CL	17	60	3	7	0	5	2	0	5
98LB789	CON	11	19	8	23	0	4	27	3	5
98LB789	FT	49	3	23	15	0	0	9	0	1
98LB789	MIX	46	18	9	14	0	13	0	0	0
Dorado	CL	3	60	23	3	0	8	3	0	0
Dorado	CON	2	11	39	29	3	0	15	2	0
Dorado	FT	68	6	11	11	0	0	2	2	0
Dorado	MIX	42	29	17	7	0	3	3	0	0
RTx2536	CL	0	59	28	11	2	0	0	0	0
RTx2536	CON	1	31	40	5	3	4	13	3	0
RTx2536	FT	47	9	19	12	0	9	5	0	0
RTx2536	MIX	31	39	19	11	0	0	0	1	0
RTx430	CL	3	57	21	3	0	13	3	0	1
RTx430	CON	17	16	24	9	0	0	27	5	3
RTx430	FT	59	10	10	12	0	0	5	0	4
RTx430	MIX	51	32	6	6	0	0	3	0	3
Sureno	CL	0	89	5	3	0	0	2	0	0
Sureno	CON	3	22	16	25	0	13	13	7	0
Sureno	FT	31	10	15	0	0	39	5	0	0
Sureno	MIX	17	47	9	13	0	7	8	0	0
Overall Mean		25	29	15	14	1	6	8	2	1

[1] Frequency of recovery (%) of the various fungal species was based on assays of 150 seeds per line/treatment combination plated on half-strength potato dextrose agar medium.
[2] Trt = treatment
[3] FS = *Fusarium semitectum*.
[4] F spp. = other *Fusarium* species.
[5] FP = *Fusarium proliferatum*.
[6] FC = *Fusarium chlamydosporum*.
[7] Alt. = *Alternaria* species.
[8] BI = *Bipolaris* spp.
[9] Others = other fungal species.

TABLE 3. Correlation of coefficients between germination and seed mycoflora of eight sorghum lines grown in College Station and inoculated at 50% anthesis with *Fusarium* (Ft), *Curvularia* (Cl), and a mixture of the two fungi in 2000 and 2001.

	Year 2000						
	Germination[1]	Ft	Cl	*F.* spp.	*Alternaria* spp.	Other species	
F. semitectum	0.48***	−0.37**	−0.21	0.25	0.23	0.77***	
Alternaria spp.	0.65***	−0.35**	−0.42**	0.47***	0.36**		
F. spp.	0.19	0.02	−0.46***	0.26			
F. semitectum	0.01	−0.42**	−0.23				
Cl	−0.44***	−0.64***					
Ft	0.07						

	Year 2001							
	Germination[1]	Ft	Cl	*F.* spp.[2]	*Alternaria* spp.	*B. polaris*	Other species	
F. semitectum	−0.12	0.01	−0.005	−0.22	−0.16	0.32*	0.003	
Bipolaris spp.	0.40	−0.28	−0.34*	0.33*	−0.18	0.65***		
Alternaria spp.	0.47***	−0.33*	−0.41**	0.33*	−0.14			
F. spp.	0.33*	−0.20	0.08	−0.16				
F. chlamydosporum	0.03	−0.42**	−0.14					
Cl	−0.01	−0.56***						
Ft	−0.52***							

[1] Germination was based on the number of seeds that germinated after 7 days out of 300 seeds per replication planted in the greenhouse.
[2] *Fusarium* spp. included *F. proliferatum*, *F.* , and other *Fusarium* species.
*, **, and *** significant at the 10%, 5%, and 1% probability levels.

tween *F. semitectum* and *F. thapsinum*, and highly significant negative correlations between *F. thapsinum* and *C. lunata* were observed.

DISCUSSION

Studies were conducted in 2000 and 2001 to assess the effects of *F. thapsinum*, *C. lunata*, and a mixture of the two fungi on germination and seed mycoflora for eight sorghum cultivars. In previous study, Prom et al. (2003) noted that fungal treatments markedly reduced seed germination rates when compared to water-sprayed controls. *C. lunata* tended to have a greater negative impact on germination in the drier season, whereas *F. thapsinum* had greater negative impact on germination in mold favorable environment (Prom et al., 2003).

Overall, *C. lunata* was the most frequently recovered fungal species across cultivars and treatments followed by *F. thapsinum* and *F. semitectum*. Chavan and Raut (1987) reported that *C. lunata* followed by *F. moniliforme* were the most frequently isolated fungal species from moldy sorghum grains in Maharashtra, India. Gan-Bobo and Dostaler (1990) noted that *C. lunata* was the most common fungal species recovered from seven cultivars of pearl millet (*Pennisetum typhoides*) seeds harvested in Niger, West Africa. Mycoflora analysis of sorghum grain from Argentina revealed *Fusarium* spp. (*F. moniliforme*, *F. subglutinans*, *F. chlamydosporum*, and *F. semitectum*), *A. alternate*, *C. lunata*, and *Bipolaris cynodontis* (Marignoni) Shoemaker as the most frequently occurring fungal species (Saubois et al., 1999). *Alternaria* spp., *Cladosprium* spp., *Colletotrichum graminicola*, *Curvularia* spp., *Drechslera* spp., *F. moniliforme*, *F. semitectum*, and other fungal species were isolated from sorghum seeds collected in Sao Paulo, Brazil (Lasca et al., 1986).

Mycoflora analysis of naturally-infected seeds from the water-sprayed controls showed *Alternaria* spp. and *F. semitectum* as the most frequently isolated fungal species followed by *C. lunata*. In naturally infected sorghum kernels, Castor and Frederiksen (1980) reported that *Alternaria* spp. were the most frequently isolated fungal species followed by *F. semitectum*, *C. lunata*, and *C. protuberata*. In this study, seeds were surface disinfected and may have reduced the number of the different fungal species, especially the external contaminants which include many *Alternaria* spp. Although, *F. thapsinum* was used in this study, it seemed to be a poor colonizer of naturally infected mature grain than *C. lunata* or *F. semitectum*. It may be that *F. thapsinum* is not

as competitive late in the season as the other fungal species. Another possibility may also be that it is not as fast growing and more internally located. Castor (1981) noted that late in the infection process, *C. lunata* was restricted from further colonization of the endosperm by the peripheral endosperm cells, whereas the colonization of the endosperm and germ by *F. moniliforme* was not restricted by the peripheral cells. The *F. moniliforme* used in previous studies may have included many *Fusarium* spp. including the species recently designated *F. thapsinum* by Klittich et al. (1997).

Germination was negatively correlated with *C. lunata* in 2000 ($P < 0.01$), and with *F. thapsinum* in 2001 ($P < 0.01$). Hepperly et al. (1982) noted negative correlations between *F. moniliforme* and *C. lunata* with seed germination. Castor (1981) and Garud et al. (2000) reported significant negative correlations between seed germination and *Fusarium* spp., but non-significant correlations between germination and *Curvularia* spp. However, Castor (1981) also noted that *C. protuberata* caused significantly higher levels of root necrosis than the control and other fungal treatments. The discrepancy between their tests and this study may be due to the different methods employed. Castor (1981) and Garud et al. (2000) used the wet paper method. This method is less stringent compared to the germination test used in this study, which recorded only those seeds that have enough energy reserve to germinate in potting media and support the first and second true leaves. The germination test used in this study gives a better correlation between seed mycoflora and field performance.

In addition, significant negative correlations between *F. semitectum* and *F. thapsinum*, and between *F. thapsinum* and *C. lunata* were detected. There also were non-significant negative correlations between *F. semitectum* and *C. lunata*. These results indicate that *F. semitectum* could interfere with the effect of these two fungal species on germination rates and also complicates the identification of grain mold resistance sources that rely on natural infestation even in mold conducive environments.

Synergistic interaction between the grain mold fungi was not frequently observed, even though grain mold is considered a disease complex involving several genera of fungi (Bandyopadhyay and Chandrashekar, 2000; Esele et al., 1995; Singh and Bandyopadhyay, 2000). This was clearly illustrated by the significant negative correlations between the two fungi and also among some of the fungal species.

In conclusion, the presence of different fungal species in mature seeds, including those that have the ability to produce mycotoxins, can severely reduce the grain quality.

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